Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11236–11240, November 1994
Medical Sciences

Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40 large tumor antigen fusion gene

(prostatic steroid-binding protein promoter/multistep tumorigenesis/hormone response element/metastasis/gene targeting)

IOANNA G. MAROULAKOU*, MIRIAM ANVER†, LISA GARRETT*, AND JEFFREY E. GREEN*‡

*Laboratory of Molecular Oncology, National Cancer Institute, P.O. Box B, Frederick, MD 21702–1201; and †Pathology/Histology Laboratory, Program Resources, Inc./DynCorp, Frederick Cancer Research and Development Center, Frederick, MD 21702–1201

Communicated by Philip Leder, June 7, 1994

ABSTRACT **A transgenic mouse model for prostate and mammary cancer has been developed in mice containing a recombinant gene expressing the simian virus 40 early-region transforming sequences under the regulatory control of the rat prostatic steroid binding protein [C3(1)] gene. Male transgenic mice develop prostatic hyperplasia in early life that progresses to adenoma or adenocarcinoma in most animals surviving to longer than 7 months of age. Prostate cancer metastases to lung have been observed. Female animals from the same founder lines generally develop mammary hyperplasia by 3 months of age with subsequent development of mammary adenocarcinoma by 6 months of age in 100% of the animals. The development of tumors correlates with the expression of the transgene as determined by Northern blot and immunohistochemical analyses. The results of these experiments demonstrate that the C3(1) regulatory region used in these experiments is useful for targeting expression to the prostate and mammary gland. To our knowledge, this experimental system is the first reported transgenic mouse model for prostate cancer. These transgenic animals offer the opportunity to study hormone response elements *in vivo* and the multistage progression from normal tissue to carcinoma in the prostate and mammary glands.**

Prostate and breast cancer are increasingly important sources of morbidity and mortality (1). Prostate cancer, the most common malignancy in males with an estimated incidence of 33% in men over the age of 50, is the second leading cause of cancer deaths in males (1). Breast cancer arises in one in nine women during their lifetime and is the leading cause of cancer deaths in women (2).

Transgenic models of neoplasias offer unique opportunities to study the biology of oncogenesis and provide systems to test experimental therapeutic approaches. Although several transgenic mouse models for mammary cancer have been developed (3–15), no transgenic mouse model of prostate cancer has been reported. Existing animal models for prostate cancer (16–24) have several significant limitations (25, 26).

We have targeted the expression of the early region of simian virus 40 (SV40) large tumor antigen (TAg) to the prostate of transgenic mice by using the 5′ flanking region of the rat prostatic steroid binding protein [C3(1)] gene (27). Two lines of transgenic mice have been propagated, both of which predictably develop prostate hyperplasia and carcinoma in males and mammary carcinoma in females. Animals from both founder lines also develop several other unusual phenotypic changes (unpublished results). The results of these studies demonstrate that the C3(1) 5′ regulatory region is capable of targeting expression to the prostate and the mammary gland. These transgenic animals are useful models for the study of multistep tumor progression in the prostate and mammary gland and may serve as experimental systems for various therapeutic regimes.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Construction of C3(1)–TAg Fusion Gene and Creation of Transgenic Mice. The 5.7-bp *Sac* I fragment from the rat C3(1) 5′ flanking region including the first exon (27, 28) was filled-in and subcloned into the blunted *Xba* I site of BlueScript SK(+) (Stratagene). The 890-bp 3′ *Nco* I–*Sac* I fragment was replaced with a 161-bp PCR-derived fragment§ in which a *Not* I site was introduced after the RNA transcriptional start site and prior to the ATG translational start site of the C3(1) gene. The *Sfi* I–*Bam*HI fragment from the early region of SV40 was inserted 3′ to the C3(1) sequences. The 7.2-kb fragment containing the C3(1)–TAg fusion was excised from plasmid sequences, purified by sucrose gradient, and injected into the pronuclei of 0.5-day FVB/N embryos (29). Transgenic offspring were identified by Southern blot and slot blot techniques utilizing a ^{32}P-labeled probe specific for SV40 sequences (30).

RNA Isolation and Analysis. RNA was extracted from tissues using the guanidine thiocyanate/cesium chloride method (30). Twenty micrograms of total RNA was separated using a denaturing 1% agarose/formaldehyde gel, transferred to nitrocellulose membranes, and hybridized to a ^{32}P-labeled SV40-specific probe (30).

Histochemical and Immunohistochemical Analyses. Gross lesions, major organs and tissues, and the accessory sex glands were collected and fixed in 4% (wt/vol) paraformaldehyde. Tissues were embedded in paraffin and sections were stained with hematoxylin/eosin (H&E) or immunostained using the Vector ABC system (Vector Laboratories) with nickel chloride-enhanced diaminobenzidine (31). Anti-TAg rabbit polyclonal antibody 115 was the kind gift of David P. Lane (University of Dundee).

RESULTS

Establishment of Transgenic Lines. Twelve transgenic founder animals were produced (Fig. 1) as determined by Southern blot analyses. Three animals died and were thus not analyzed. Two animals have survived to 18 months of age without apparent abnormalities and have not transmitted the transgene to their offspring. Of the remaining seven founder animals, two were able to transmit the transgene to offspring (designated lines C and L).

Abbreviations: SV40, simian virus 40; TAg, large tumor antigen; H&E, hematoxylin/eosin.

‡To whom reprint requests should be addressed.

§The sequence of the 5′ PCR primer used is CTCTTATGTTTCCTGGTGCAGTGCCATGGTA; the sequence of the 3′ PCR primer used is AAACACCAGCTTCATGTTGCGGCCGCCCCTGTGGGTTGTT.

FOUNDER MICE				PHENOTYPE									
Female	Male	Copy No.	Age at Death	Prostate hyperplasia	Breast adenocarcinoma	Chondrodysplasia	Sal. gland proliferative lesions	Nasal turbinate proliferative lesions	Harderian hyperplasia	Thyroid proliferative lesions	Osteosarcoma	Lung carcinoma
A*		2	1.5 yrs									
E		10	9 wks			— N.D. —						
C		6	20 wks		+							
L		4	16 wks		+	+	+	+	+			+
F		50	12 wks		+	+	+	+	+	+[c]		
J		10	4.5 wks			+	+	+	+	+[a]		
	G	40	7 wks			+	+	+	+	+[b]		
	K	5	10 wks	+		+	+	+			+	
	I	4	6 wks	+		+	+	+	+	+[a]	+	+
	D	40	5.5 wks			— N.D. —						
	B*	1	–			— N.D. —						
	M	>100	1 day			— N.D. —						

a = hyperplasia, b = adenoma, c = carcinoma
N.D.: Not determined, * No transmission

FIG. 1. Phenotypic abnormalities of PRP-3 transgenic founder mice.

Phenotypic Abnormalities of Founder Mice. Of the eight founder animals analyzed, three of five females developed mammary adenocarcinoma and two of three male animals developed prostate hyperplasia. Phenotypic abnormalities in most of the founder mice were significant (Fig. 1) with only two founder animals surviving longer than 20 weeks; these two founders did not transmit the transgene and, presumably, were mosaic for the transgene. The severity of the phenotypes appeared to generally correlate with the transgene copy number, which ranged from 1 to >100 copies per genome. Other lesions included the development of osteosarcoma; proliferative lesions of the thyroid, salivary glands, and nasal epithelium; and an unusual form of chondrodysplasia (unpublished results).

Phenotypic Abnormalities in Two Lines of Transgenic Mice. Two founder animals, C and L, were able to transmit the transgene to their offspring. The development of pathologic changes was extensively studied in these two lines of transgenic mice. The survival curves for animals from the C line (Fig. 2) demonstrate a clear distinction between male and female animals.

Prostate. In male mice, hyperplastic changes in the epithelium of the dorsal/ventral regions of the prostate were noted as early as 3 months of age. Atypical epithelial hyperplasia (Fig. 3 *A* and *B*) consisted of pseudostratified or piled-up cells with hyperchromatic spindle-shaped nuclei, basophilic cytoplasm, and a high nuclear/cytoplasmic ratio. These are dysplastic lesions. The basement membrane was intact. Adenomas occurred in about one-third of animals between 6 and 8 months of age. Papillary adenomas were composed of exophytic papillary projections of epithelial cells extending into acinar lumens on delicate fibrovascular cores (Fig. 3*C*). Epithelium had a similar appearance to that described for atypical hyperplasia. Male animals survived longer than females, up to 11 months, with the majority of males developing prostate adenocarcinoma after 8 months of age.

Adenocarcinomas were composed of cells with hyperchromatic pleomorphic nuclei with many atypical mitotic figures and basophilic cytoplasm with poorly defined borders. Tumor cells formed small irregular acinar structures lined by piled-up epithelium or were arranged in sheets and/or clumps (Fig. 3 *D*, *E*, and *G*). In some areas of the tumors, cells formed cribriform glandular structures with disorganized hyperchromatic cells (Fig. 3*F*). Adenocarcinomas were locally invasive and one adenocarcinoma metastasized to the lung. Desmoplasia of the type seen in human and rat prostatic malignancies was not present.

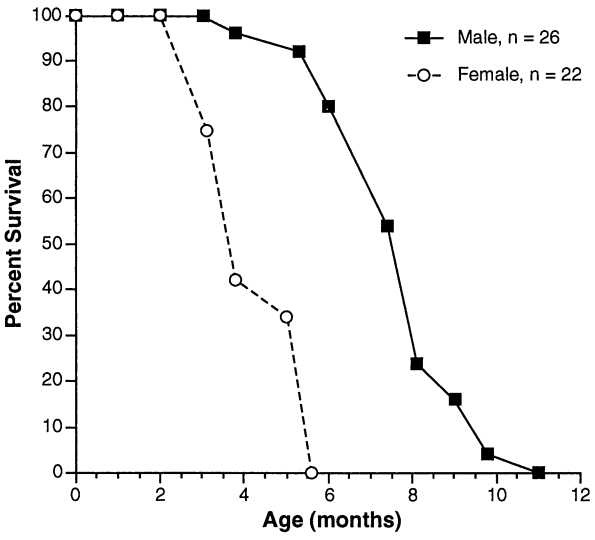

FIG. 2. Survival curve of PRP-3 transgenic mice.

Mammary Gland. Two-thirds of female mice developed atypical hyperplasia in ducts and acini by 3 months of age (Fig. 4*B*). When acini were involved, lesions resembled those classified as hyperplastic alveolar nodules (32). Female mice succumb by 6 months of age with the universal development of multifocal mammary adenocarcinoma with occasional evidence of metastatic involvement to the lung. Microscopically, adenocarcinomas could be classified as Dunn type B tumors (32). Tumors in PRP-3 transgenic mice were solid, composed of small irregular glandular structures in sheets or clumps of poorly differentiated epithelial cells with vesicular-to-hyperchromatic round nuclei, basophilic cytoplasm with poorly defined borders, high nuclear/cytoplasmic ratio, and high mitotic index (Fig. 4 *D–F*). Nonparous females typically developed mammary adenocarcinoma at about 4 months of age. The development of mammary adenocarcinoma appeared to be accelerated in animals that became pregnant.

Mice of the L series developed mammary or prostate hyperplasia, but most appeared to die from nasal epithelial tumors between 5 and 8 months of age, prior to the development of adenocarcinoma of the prostate or mammary gland. Prostate and mammary adenocarcinomas did develop in the oldest surviving L mice. No spontaneous prostate or mammary tumors were observed in nontransgenic FVB/N mice.

Expression of SV40 TAg and Tumor Formation. The development of prostate and mammary lesions correlates with the expression of the SV40 transgene. Northern blot analysis of various tissues from female founder animal C demonstrated that TAg was expressed in the mammary tumors (Fig. 5*A*, lanes 7–9), but not in tissues with apparently normal histology (Fig. 5*A*, lanes 1–6). The expression pattern in C-line descendant females was similar to that of the founder animal (data not shown). Transgenic females from the L line also demonstrated high levels of TAg in mammary tumors (Fig. 5*A*, lane 15). Expression of TAg was localized using immunocytochemical analyses. Strong staining was observed in apparently normal mammary ductal cells prior to

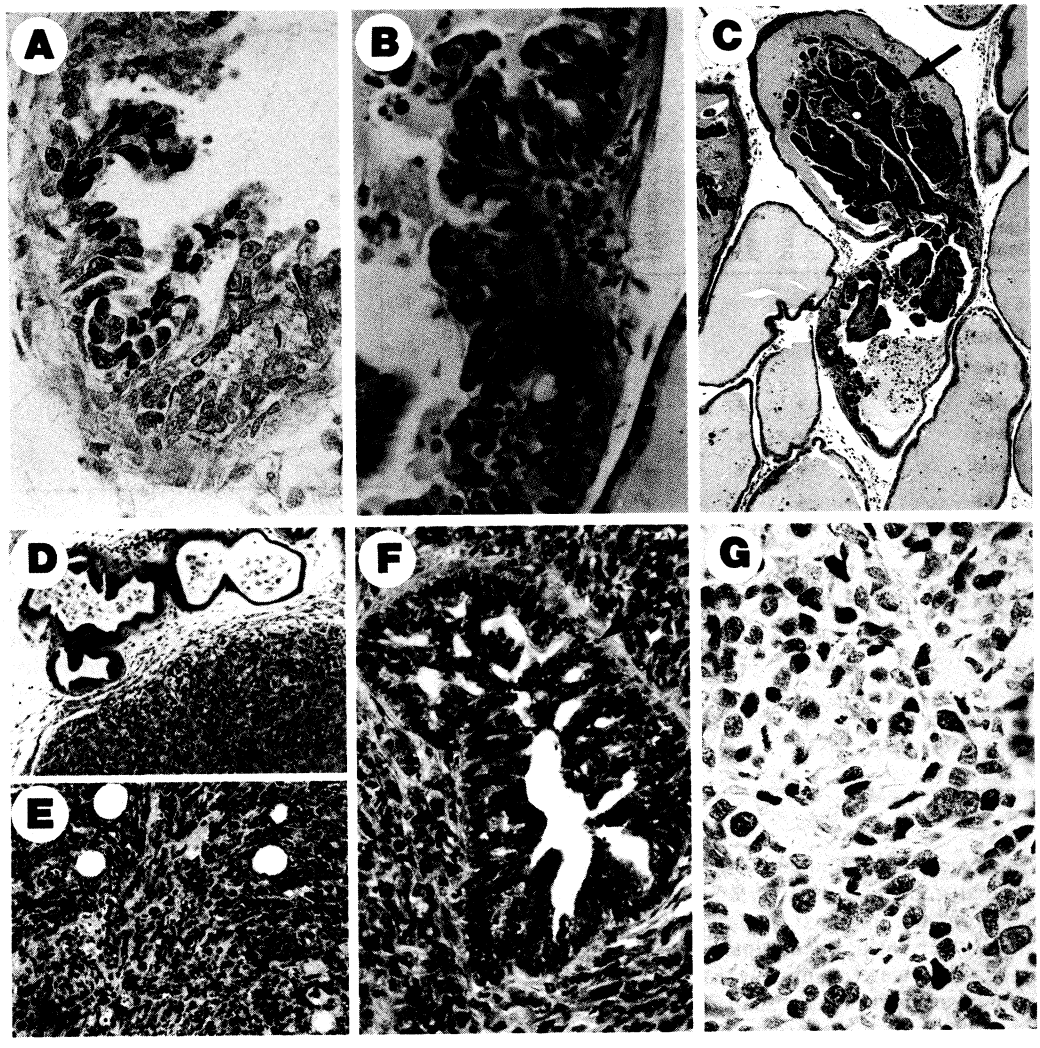

FIG. 3. Transgenic prostate lesions. (*A*) Region of prostate epithelium with scattered cell nuclei positive for TAg. (Immunohistochemistry; ×630.) (*B*) Atypical hyperplasia, dorsolateral prostate. Epithelial cells are piled-up or pseudostratified with spindle-shaped nuclei. (H&E; ×630.) (*C*) Adenoma (arrow), dorsal prostate. Papillary projection in acinar lumen of atypical epithelium on delicate fibrovascular cores. (H&E; ×100.) (*D*) Adenocarcinoma, ventral prostate. The tumor is composed of sheets of poorly differentiated cells. (H&E; ×100.) (*E*) Adenocarcinoma, ventral prostate. Sheets and clumps of poorly differentiated cells with scattered glandular structures. (H&E; ×200.) (*E*) Adenocarcinoma, ventral prostate. Cribriform glandular structure (arrow) within area of spindle-shaped tumor cells. Numerous mitotic figures. (H&E; ×400.) (*G*) Adenocarcinoma with scattered nuclei staining darkly for TAg. (Immunohistochemistry; ×630.)

the development of hyperplasia or neoplasia (Fig. 4*A*). TAg was also detected in the mammary carcinoma cells (Fig. 4 *D* and *F*). TAg was detected in the nuclei, as expected, but the intensity varied from cell to cell. TAg was not detectable in all tumor cells by immunocytochemistry. Immunohistochemical analysis for TAg was negative in tissues from nontransgenic control animals (data not shown). Similarly, male animals from the C and L lines expressed TAg transcripts in prostate carcinomas (Fig. 5*B*, lanes 3 and 13) and salivary gland (Fig. 5*B*, lanes 4 and 10) but not in brain, kidney, lung, liver, spleen, and heart (Fig. 5*B*, lanes 1, 5–9, 11, 12, 14, 16, and 17). A very low level of TAg was occasionally detectable in the testes (data not shown). Immunocytochemical analyses revealed TAg expression in scattered apparently normal-appearing prostate epithelial cells. Expression levels varied from cell to cell in regions of prostate hyperplasia (Fig. 3*B*) and in the adenocarcinomas (Fig. 3*H*).

DISCUSSION

The rat C3(1) 5′ regulatory region has been used to direct the expression of SV40 TAg to the prostate and mammary glands, resulting in a transgenic model for the study of multistage oncogenesis in these tissues. Transgenic mice can be propagated that consistently develop proliferative prostate and mammary lesions that progress over time to adenocarcinoma, making this an attractive model for the study of multistage progression of tumor development in these tissues.

Histologically, the transgenic prostate and mammary tumors share similarities to the human counterparts. Prostate adenocarcinoma in the transgenic mice is similar to the more poorly differentiated variants of human prostate carcinoma (33). The atypical mammary hyperplasias and carcinomas observed in the female transgenic mice involve small ducts and acini, which share similarities to certain types of human proliferative breast lesions, including atypical hyperplasia and *in situ* lobular carcinoma (34).

Based upon the immunohistochemical studies of prostate and mammary lesions, it does not appear that all TAg-expressing cells are hyperplastic or develop carcinoma (Figs. 3*B* and 4*A*). While the expression of TAg is necessary for tumor development in these animals, it does not appear sufficient by itself for complete transformation. Evidently, other genetic events are required for complete transformation to occur.

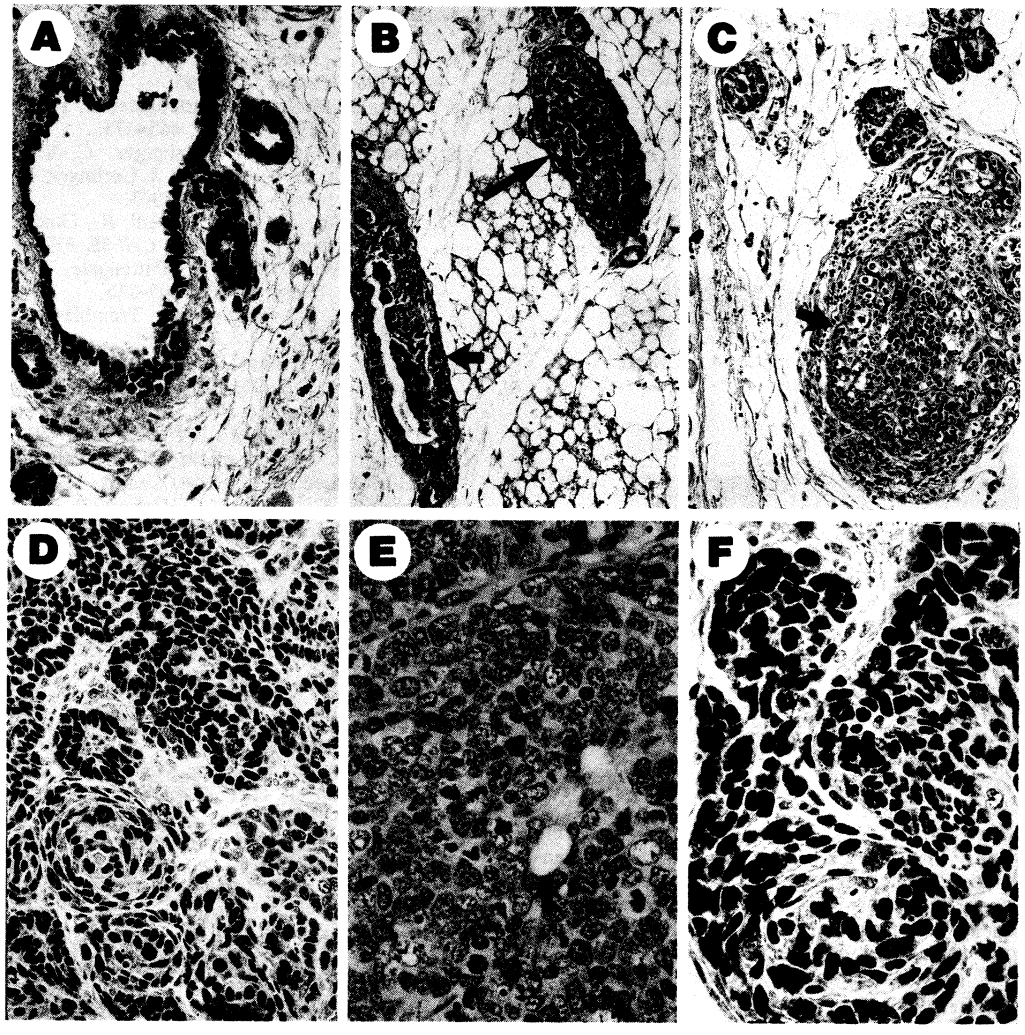

FIG. 4. Transgenic mammary gland lesions. (*A*) Nonproliferative ducts with positive dark nuclear immunostaining for TAg. (×400.) (*B*) Duct (small arrow) and acinus (large arrow) with atypical epithelial hyperplasia. (H&E; ×100.) (*C*) Microscopic adenocarcinoma (arrow). Irregular small acini and sheets of cells lack polarity with loss of basement membrane integrity. (H&E; ×100.) (*D*) Adenocarcinoma with numerous darkly stained nuclei positive for TAg. (Immunohistochemistry; ×400.) (*E*) Adenocarcinoma composed of irregular acini (arrow) and sheets of anaplastic epithelium. Numerous mitotic figures. (H&E; ×630.) (*F*) Adenocarcinoma with numerous darkly stained nuclei positive for TAg. (Immunohistochemistry; ×630.)

Although transgenic models for mammary carcinoma have been developed previously (3–15), to our knowledge, this is the first transgenic model of prostate carcinoma. Overexpression of the *int-2* gene under control of the mouse mammary tumor virus long terminal repeat in another transgenic system leads to benign prostatic hyperplasia, but these lesions do not progress to malignancy (11). Another rodent model for studying prostate cancer has been developed using retroviral infection of an embryonic organ reconstitution culture system (21), but this system has several limitations. Prostate cancer has been induced in rats using chemical carcinogens (17, 18, 20, 23, 24). These systems are limited by the required use of strong carcinogens and nonphysiologic hormone manipulations. For the Lobund–Wistar rat model, tumors primarily arise in the seminal vesicles and infrequently in the dorsolateral prostate (26), the region that is most relevant to human prostate cancer.

The C3(1)–TAg construct appears to be highly responsive to both male and female sex hormones. Previous studies have identified several hormone response elements within the 5′ region, first exon, and intron of the rat C3(1) gene (35). When introduced into the germ line of mice, a 9-kb intact C3(1) gene was shown to direct expression of the rat prostatein protein specifically to the prostate in transgenic mice (36). Our construct only utilized the hormone response elements reported to be within the 5′ region and first exon (35). Our results indicate that the 5′ C3(1) region we used lacks important regulatory information to limit expression exclusively to the prostate. However, the previous transgenic experiments demonstrating prostate-specific expression of the entire C3(1) gene may not have detected low levels of expression in other tissues due to the limitation of sensitivity by Northern blot analysis. It is possible that the use of TAg in our construct greatly increases the sensitivity of scoring where the C3(1) promoter is expressed since low levels of expression may lead to transformation of those tissues. Our construct also expressed in several other tissue types, including the thyroid, salivary gland, and cartilage (unpublished data).

Recently, the rat probasin gene promoter (PB) has been reported to direct the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene specifically to the prostate in transgenic mice in a developmentally and hormonally regulated fashion (37). The C3(1) regulatory region utilized in our experiments appears to be more promiscuous than the PB promoter, although the cointegration of heterologous chicken lysozyme matrix attachment elements with the C3(1)–TAg construct might also suppress ectopic expres-

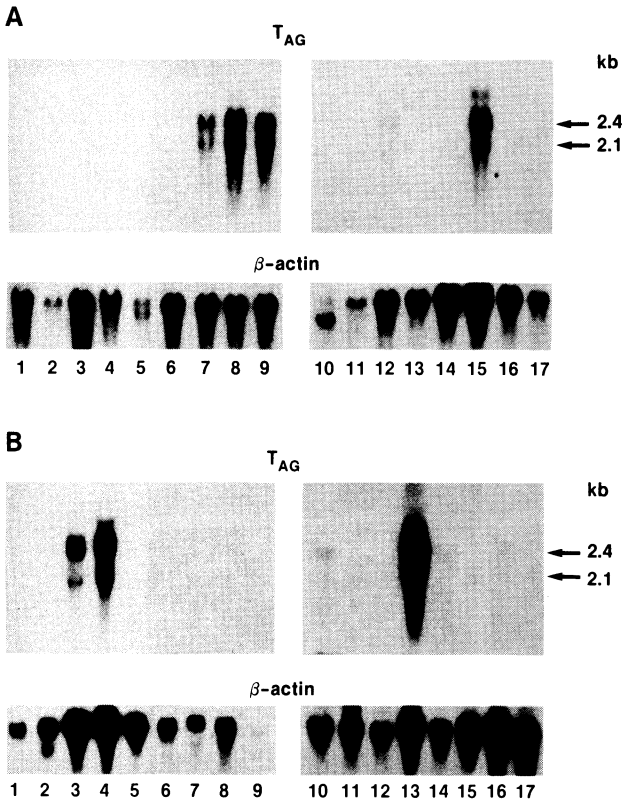

FIG. 5. Northern blot analyses of RNA expression in transgenic mice. The arrowheads show the 2.4-kb and 2.1-kb transgene transcripts of the SV40 small tumor antigen and TAg, respectively. (*A*) Female. Lanes: 1–9, founder animal C; 10–17, founder line L descendants; 1, kidney; 2, salivary gland; 3, lung; 4, liver; 5, muscle; 6, spleen; 7–9, mammary tumors; 10, muscle; 11, liver; 12, lung; 13, brain; 14, spleen; 15, mammary tumor; 16, kidney; 17, heart. (*B*) Male. Lanes: 1–9, descendant of C founder; 10–17, descendant of founder L; 1, brain; 2, testis; 3, prostate; 4, salivary gland; 5, kidney; 6, lung; 7, liver; 8, spleen; 9, heart; 10, salivary gland; 11, kidney; 12, brain; 13, prostate/seminal vesicle tumor; 14, heart; 15, testis; 16, lung; 17, spleen.

sion of the transgene, as was observed in the PB–CAT transgenics (37). The C3 (1)-TAg transgenic system provides a model to study hormone response elements *in vivo* and to define determinants of androgen- and estrogen-specific regulation required for gene targeting to the prostate and mammary glands. These transgenic animals provide a model to study the TAg-induced multistage progression of tumors in androgen and estrogen responsive tumors.

Note Added in Proof. We have recently learned that another transgenic model of prostate cancer has been developed by using a PB-TAg construct (N. M. Greenberg, personal communication).

We are grateful to Dr. T. Papas for support, Dr. J. Ward and Dr. R. Newburgh for critical review of the manuscript, Dr. M. Parker for providing the C3(1) genomic clone, Dr. D. Lane for antibody to TAg, Dr. J. Brady for SV40 DNA, and Karen Cannon for preparation of the manuscript.

1. Hanks, G. E., Myers, C. E. & Scardino, P. T. (1993) in *Cancer: Principles and Practice of Oncology*, eds. DeVita, V. T., Jr., Hellman, S. & Rosenberg, S. A. (Lippincott, Philadelphia), 4th Ed., pp. 1073–1113.
2. Harris, J. R., Morrow, M. & Bonadonna, G. (1993) in *Cancer: Principles and Practice of Oncology*, eds. DeVita, V. T., Jr., Hellman, S. & Rosenberg, S. A. (Lippincott, Philadelphia), 4th Ed., pp. 1264–1332.
3. Stewart, T. A., Pattengale, P. K. & Leder, P. (1984) *Cell* **38**, 627–637.
4. Leder, A., Pattengale, P. K., Kuo, A., Stewart, T. A. & Leder, P. (1986) *Cell* **45**, 485–495.
5. Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace, A. & Leder, P. (1987) *Cell* **49**, 465–475.
6. Andres, A. C., Schonenberger, C. A., Groner, B., Hennighausen, L., LeMur, M. & Gerlinger, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1299–1303.
7. Tsukamoto, A. S., Grosschedl, R., Guzman, R. C., Parslow, T. & Varmus, H. E. (1988) *Cell* **55**, 619–625.
8. Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R. & Leder, P. (1988) *Cell* **54**, 105–115.
9. Bouchard, L., Lamarre, L., Tremblay, P. J. & Jolicoeur, P. (1989) *Cell* **57**, 931–936.
10. Matsui, Y., Halter, S. A., Holt, J. T., Hogan, B. L. M. & Coffey, R. J. (1990) *Cell* **61**, 1147–1155.
11. Muller, W. J., Lee, F. S., Dickson, C., Peters, G., Pattengale, P. & Leder, P. (1990) *EMBO J.* **9**, 907–913.
12. Sandgren, E. P., Letteke, N. C., Palmiter, R. D., Brinster, R. L. & Lee, D. C. (1990) *Cell* **61**, 1121–1135.
13. Jhappan, C., Stahle, C., Harkins, R. N., Fausto, N., Smith, G. H. & Merlino, G. T. (1990) *Cell* **61**, 1137–1146.
14. Jhappan, C., Gallahan, D., Stahle, C., Chu, E., Smith, G. H., Merlino, G. & Callahan, R. (1992) *Genes Dev.* **6**, 345–355.
15. Tzeng, Y.-J., Guhl, E., Graessmann, M. & Graessmann, A. (1993) *Oncogene* **8**, 1965–1971.
16. Pollard, M. & Luckert, P. H. (1975) *J. Natl. Cancer Inst.* **54**, 643–649.
17. Pollard, M. & Luckert, P. H. (1987) *Prostate* **11**, 219–227.
18. Pollard, M., Luckert, P. H. & Snyder, D. L. (1989) *Cancer Lett.* **45**, 209–212.
19. Beckman, W. C., Jr., Jacokes, A. L., Camps, J. L., Jr., Cook, R. L. & Siegal, G. P. (1987) *Am. J. Pathol.* **128**, 566–572.
20. Waalkes, M. P., Rehm, S., Riggs, C. W., Bare, R. M., Devor, D. E., Poirier, L. A., Wenk, M. L., Henneman, J. R. & Balaschak, M. S. (1988) *Cancer Res.* **48**, 4656–4663.
21. Thompson, T. C., Southgate, J., Kitchener, G. & Land, H. (1989) *Cell* **56**, 917–930.
22. Ward, J. M., Reznik, G., Stinson, S. F., Lattuada, C. P., Longfellow, D. G. & Cameron, T. P. (1980) *Lab. Invest.* **6**, 517–522.
23. Ito, N., Shirai, T., Tagawa, Y., Nakamura, A. & Fukushima, S. (1988) *Cancer Res.* **48**, 4629–4632.
24. Shirai, T., Tamano, S., Kato, T., Iwasaki, S., Takahashi, S. & Ito, N. (1991) *Cancer Res.* **51**, 1264–1269.
25. Buttyan, R. & Slawin, K. (1993) *Cancer Metastasis Rev.* **12**, 11–19.
26. Cohen, M. B., Heidger, P. M. & Lubaroff, D. M. (1994) *Cancer Res.* **54**, 626–628.
27. Parker, M. G., White, R., Hurst, H., Needham, M. & Tilly, R. (1983) *J. Biol. Chem.* **258**, 12–15.
28. Hurst, H. C. & Parker, M. G. (1983) *EMBO J.* **2**, 769–774.
29. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
30. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
31. Hsu, S. M. & Soban, E. (1982) *J. Histochem. Cytochem.* **30**, 1079–1082.
32. Squartini, F. (1979) in *Pathology of Tumours in Laboratory Animals, Volume 2, Tumors of the Mouse*, ed. Turusov, V. S. (IARC, Lyon, France), pp. 43–90.
33. Gleason, D. F. (1977) in *Urologic Pathology: The Prostate*, ed. Tannenbaum, M. (Lee & Febiger, Philadelphia), pp. 171–198.
34. McDivitt, R. W. (1990) in *Anderson's Pathology*, ed. Kissane, J. M. (Mosby, St. Louis), Vol. 2, pp. 1726–1750.
35. Tan, J., Marschke, K. B., Ho, K.-C., Perry, S. T., Wilson, E. M. & French, F. S. (1992) *J. Biol. Chem.* **267**, 4456–4466.
36. Allison, J., Zhang, Y. L. & Parker, M. G. (1989) *Mol. Cell. Biol.* **9**, 2254–2257.
37. Greenberg, N. M., DeMayo, F. J., Sheppard, P. C., Barrios, R., Lebovitz, R., Finegold, M., Angelopoulou, R., Dodd, J. G., Duckworth, M. L., Rosen, J. M. & Matusik, R. J. (1994) *Mol. Endocrinol.* **8**, 230–239.